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Research Article

Morphological and molecular data reveal *Cerrena caulinicystidiata* sp. nov. and *Polyporus minutissimus* sp. nov. in Polyporales from Asia

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Abstract

Two new species of Polyporales, *Cerrena caulinicystidiata* and *Polyporus minutissimus*, are illustrated and described on the basis of morphological studies and phylogenetic analyses from southern China and Vietnam. *C. caulinicystidiata* is characterized by annual, resupinate, sometimes effused-reflexed basidiocarps, greyish orange to brownish orange pore surface, irregular pores (3–8 per mm), a trimitic hyphal system, pyriform to ventricose cystidia, and subglobose basidiospores 3.2–4.5 × 2.8–3.5 µm in size. *P. minutissimus* is characterized by annual, solitary, fan-shaped with a depressed center or infundibuliform basidiocarps, obvious black stipe, cream to buff yellow pileal surface with glabrous, occasionally zonate and radially aligned stripes, angular pores (6–9 per mm), a dimitic hyphal system, and cylindrical basidiospores, 5–9.2 × 2.2–4 µm. Detailed descriptions and illustrations of the two new species are provided. The differences between the two new species and their morphologically similar and phylogenetically related species are discussed.

Key words: Cerrenaceae, phylogeny, Polyporaceae, taxonomy, wood-decaying fungi

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Introduction

The order Polyporales presents a great diversity of basidiocarp types and hymenophore configurations (Binder et al. 2013). It is an important group of fungi, as Polyporales species can cause wood-decay and thus play an essential role in the carbon cycle. In addition, some species of Polyporales may have medicinal properties (Dai 1996, 1999, 2012; Dai et al. 2009). The order has long been the subject of research on taxonomic diversity, distribution patterns, and ecological functions (Hibbett et al. 2014). As of early 2024, more than 1,800 species are recognized in the order (Martinez et al. 2004; Martinez et al. 2009; Kirk et al. 2008; Grigoriev et al. 2013; Zhao et al. 2015; Justo et al. 2017). Due to its great diversity, the order is intensively studied worldwide (Justo et al. 2017).

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Cerrena Gray is the type genus of Cerrenaceae within the Polyporales (Ryvarden and Gilbertson 1993; Justo et al. 2017), and it is typified by *C. unicolor* (Bull.) Murrill. It is widely distributed throughout the world. The genus is characterized by resupinate, effused-reflexed or pileate basidiocarps, irregular hymenophore, dimitic or trimitic hyphal systems, cylindric to ellipsoid basidiospores, and white rot (Ryvarden and Gilbertson 1993; Ryvarden et al. 2022). The genus *Cerrena* published in 1821 has priority above *Trametes* Fr., published in 1838, and the two genera were considered as a single taxon in several studies. Thus, a huge number of new combinations in *Cerrena*. (Cunningham and Cunningham1965; Gilbertson and Ryvarden 1987; Corner 1989; Ryvarden and Melo 2022). However, Ryvarden (1991) recommended to keep *Cerrena* as a separate genus based on their anatomical characters. According to Index Fungorum (http://www.indexfungorum.org) and Yuan (2014), *Cerrena* currently comprises around 10 species.

Polyporus P. Micheli ex Adans., the type genus of the Polyporaceae, is a well-known polypore genus (Gilbertson and Ryvarden 1987). Given that Micheli (1729) did not originally select a type species for *Polyporus*, there is no consensus on the selection of the type. Since Donk (1933) selected *P. tuberaster* (Jacq. ex Pers.) Fr. as the type species, this lectotype was accepted by most subsequent mycologists (Cunningham and Cunningham 1965; Singer 1986; Niemelä and Kotiranta 1991; Ryvarden 1991; Sotome et al. 2008; Ji et al. 2022). Morphologically, *Polyporus* is a heterogeneous genus including many species belonging to six morphological groups described by Núñez and Ryvarden (1995), viz, the *Polyporus* group, the *Favolus* group (= *Favolus* Fr.), the *Melanopus* group (= *Melanopus* Pat.), the *Polyporellus* group (= *Polyporellus* Karst.), the *Admirabilis* group, and the *Dendropolyporus* group (= *Dendropolyporus* (Pouz.) Jülich). Phylogenetically, *Polyporus* s. str. is known as a polyphyletic genus (Krüger et al. 2006; Sotome et al. 2008, 2011; Ji et al. 2022). Phylogenetic analyses of *Polyporus* did not conform to the six morphological groups, for which further in-depth study of the group is needed (Sotome et al. 2008; Zhou et al. 2016).

During investigations on wood-decaying polypores from South China and Vietnam, specimens that morphologically fit the definitions of *Cerrena* and *Polyporus* were collected. Phylogenetically, these samples formed two distinct lineages within *Cerrena* and *Polyporus*, respectively, and they are different from their morphologically similar and phylogenetically related species. Therefore, we describe and illustrate two new species, *Cerrena caulinicystidiata* sp. nov. and *Polyporus minutissimus* sp. nov. within the Polyporales on the basis of morphological studies and phylogenetic analyses.

Materials and methods

Morphological studies

The studied specimens are deposited in the Fungarium of Beijing Forestry University (BJFC) and the Institute of Applied Ecology of the Chinese Academy of Sciences (IFP). Macro-morphological descriptions were based on field notes and voucher herbarium specimens. Microscopic measurements and drawings were made from slides prepared from voucher tissues and stained with Cotton Blue and Melzer's reagent. The following abbreviations were used: KOH = 5%

potassium hydroxide; CB = Cotton Blue; CB+ = cyanophilous in Cotton Blue; CB– = acyanophilous in Cotton Blue; IKI = Melzer's; IKI– = neither amyloid nor dextrinoid in Melzer's reagent; L = mean basidiospore length (arithmetic average of basidiospores); W = mean basidiospore width (arithmetic average of basidiospores); Q = variation in the L/W ratios between specimens studied; n (a/b) = number of basidiospores (a) measured from the given number of specimens (b). When we present basidiospore size variation, 5% of measurements were excluded from each end of the range. These excluded values are given in parentheses. Special color terms follow Anonymous (1969) and Petersen (1996).

DNA extraction and sequencing

A CTAB rapid plant genome extraction kit (Aidlab Biotechnologies, Co., Ltd., Beijing, China) was used to obtain DNA products from voucher specimens following the manufacturer's instructions with some modifications (Wu et al. 2020, 2022). The following primer pairs were used to amplify the DNA: ITS5 and ITS4 for the internal transcribed spacer (ITS) region (White et al. 1990) and LR0R and LR7 for the nuclear large subunit (nLSU) rDNA gene (Vilgalys and Hester 1990).

The procedures for DNA extraction and polymerase chain reaction (PCR) used in this study were the same as described by Wu et al. (2022). The PCR products were purified and sequenced by Beijing Genomics Institute (BGI), China. All newly generated sequences in this study were deposited in GenBank (Sayers et al.2024; http://www.ncbi.nlm.nih.gov/genbank/) and listed in Table 1.

Phylogenetic analysis

Phylogenetic trees of *Cerrena* and *Polyporus* were constructed using the two concatenated ITS1-5.8S-ITS2-nLSU sequences dataset, respectively, and phylogenetic analyses were performed with Maximum Likelihood (ML) and Bayesian Inference (BI) methods. New sequences generated in this study and reference sequences retrieved from GenBank (Table 1) were partitioned to ITS1, 5.8S, ITS2, nLSU and then aligned separately using MAFFT v.74 (Katoh et al. 2019; http://mafft.cbrc.jp/alignment/server/) with the G-INS-I iterative refinement algorithm and optimised manually in BioEdit v.7.0.5.3 (Hall 1999). The separate alignments were then concatenated using PhyloSuite v.1.2.2 (Zhang et al. 2020). Unreliably aligned sections were removed before the analyses, and efforts were made to manually inspect and improve the alignment. The data matrix was edited in Mesquite v3.70. *Irpex latemarginatus* (Durieu & Mont.) C.C. Chen & Sheng H. Wu was used as an outgroup in the phylogenetic analysis of *Cerrena* (Parmasto and Hallenberg 2000). *Trametes conchifer* (Schwein.) Pilát, *T. elegans* (Spreng.) Fr. and *T. polyzona* (Pers.) Justo were selected as outgroups in the phylogenetic analysis of *Polyporus* (Ji et al. 2022). The final alignments and the retrieved topologies were deposited in TreeBASE (http://www.treebase.org) under accessions 31102, 31103.

RAxML 7.2.8 was used to infer ML trees for both datasets with the GTR+I+G model of site substitution, including estimation of Gamma-distributed rate heterogeneity and a proportion of invariant sites (Stamatakis 2006). The branch support was evaluated with a bootstrapping method of 1,000 replicates (Hillis and Bull 1993).

Table 1. Taxa information and GenBank accession numbers of the sequences used in this study.

Species	Specimen No.	Country	ITS	LSU
Cerrena albocinnamomea	Miettinen 10511	China	OR262168	OR262168
Cerrena albocinnamomea	NIBRFG0000102423	South Korea	FJ821532	–
Cerrena albocinnamomea	Dai 12892	China	KC485522	KC485539
Cerrena albocinnamomea	KUC20121102-06	South Korea	KJ668561	–
Cerrena caulinicystidiata	**Yuan 12664**	**Vietnam**	**MT269762**	**MT259328**
Cerrena caulinicystidiata	**Yuan 12666**	**Vietnam**	**MT269763**	**MT259329**
Cerrena caulinicystidiata (*Cerrena* sp. 1)	BJ2-11	China	KX527879	–
Cerrena caulinicystidiata (*Cerrena* sp. 1)	G1669	China	MK247953	–
Cerrena caulinicystidiata (*Cerrena* sp. 1)	Otu0185	China	MT908560	–
Cerrena caulinicystidiata*	**Wu 661**	**China**	**PP035831**	**PP035828**
Cerrena cystidiata	548/17	Brazil	MZ649034	MZ649034
Cerrena gilbertsonii	JV 1609/29	Guadeloupe	OR262202	–
Cerrena gilbertsonii	Vandevender 94-144	Mexico	OR262171	OR262171
Cerrena multipileata	JV 1407/63	Costa Rica	OR262201	OR262201
Cerrena multipileata	Ryvarden 43881	Costa Rica	OR262155	OR262155
Cerrena multipileata	Kout A36	Guatemala	OR262203	–
Cerrena sp. 2	F12	China	OP022000	–
Cerrena sp. 2	7-SU-3-B-77(M)-B	Indonesia	KJ654531	–
Cerrena sp. 2	NTOU5117	Taiwan	MN592928	–
Cerrena unicolor	B2	Antarctica	HM589361	–
Cerrena unicolor	D.T6.5_2	Argentina	MH019790	–
Cerrena unicolor	CBS 154.29	Canada	MH855029	–
Cerrena unicolor	He6082	China	OM100740	OM083972
Cerrena unicolor	GSM-10	China	JQ798288	–
Cerrena unicolor	Han 849	China	MW467890	–
Cerrena unicolor	CU2	Czech	FJ821536	–
Cerrena unicolor	H:Otto Miettinen 9443	Finland	FN907915	FN907915
Cerrena unicolor	MUT<ITA_:5063	Italy	MK581063	–
Cerrena unicolor	FCG-1937	Japan	LC415531	–
Cerrena unicolor	Pertti Uotila 47558 (H)	Kyrgyzstan	OR262167	–
Cerrena unicolor	Feketic	Serbia	MW485440	–
Cerrena unicolor	KA17-0024	South Korea	MN294859	–
Cerrena unicolor	3115	Sweden	JN710525	JN710525
Cerrena unicolor	CUZFVG179	Turkey	MK120293	–
Cerrena unicolor	K(M):249944	UK	MZ159683	–
Cerrena unicolor	FD-299	USA	KP135304	KP135209
Cerrena unicolor	TASM: YG/PS79	Uzbekistan	MT526291	–
Cerrena zonata	Gates 2008-4-17 (H)	Australia	OR262160	OR262160
Cerrena zonata	Otto Miettinen 9773 (H)	China	OR262157	OR262157
Cerrena zonata	Otto Miettinen 9889 (H)	China	OR262158	OR262158
Cerrena zonata	Otto Miettinen 13798 (H)	Indonesia	OR262166	OR262166
Cerrena zonata	WS36_1_2_B_As	Japan	LC631683	–
Cerrena zonata	PDD:95790	New Zealand	HQ533016	–

Species	Specimen No.	Country	ITS	LSU
Cerrena zonata	KA17-0224	South Korea	MN294861	–
Cerrena zonata	LE-BIN 4492	Vietnam	OP985107	–
Datroniella scutellata	RLG9584T	USA	JN165004	JN164792
Datroniella tropica	Dai 13147	China	KC415181	KC415189
Echinochaete brachypora	TFM:F 24996	Japan	AB462321	AB462309
Echinochaete russiceps	TFM:F 15716	Japan	AB462310	AB368065
Echinochaete russiceps	TFM:F 24250	Japan	AB462313	AB462301
Favolus acervatus	Cui 11053	China	KU189774	KU189805
Favolus acervatus	Dai 10749b	China	KX548953	KX548979
Favolus gracilisporus	Cui 4292	China	KX548970	KX548992
Favolus gracilisporus	Li 1938	China	KX548971	KX548993
Hexagonia glabra	Dai 10691	China	JX569733	JX569750
Hexagonia tenuis	Cui 8468	China	JX559277	JX559302
Irpex latemarginatus	Dai 8289	China	KY131835	–
Lentinus longiporus	DAOM:229479	Canada	AB478880	LC052217
Lentinus longiporus	WD2579	Japan	AB478879	LC052218
Lentinus substrictus	Wei 1582	China	KU189767	KU189798
Lentinus substrictus	Wei 1600	China	KC572022	KC572059
Microporus affinis	Cui 7714	China	JX569739	JX569746
Microporus flabelliformis	Dai 11574	China	JX569740	JX569747
Mycobonia flava	CulTENN10256	Costa Rica	AY513570	AJ487934
Mycobonia flava	TENN59088	Argentina	AY513571	AJ487933
*Neodatronia gaoligongensis**	Cui 8055	China	JX559269	JX559286
*Neodatronia sinensis**	Dai 11921	China	JX559272	JX559283
Neofavolus cremeoalbidus	Cui 12412	China	KX899982	KX900109
*Neofavolus cremeoalbidus**	TUMH:50009	Japan	AB735980	AB735957
Neofavolus mikawai	Cui 11152	China	KU189773	KU189804
Neofavolus mikawai	Dai 12361	China	KX548975	KX548997
Physisporinus lineatus	JV_1008_18	Costa Rica	OM669902	–
Physisporinus lineatus	JV_1407_37	Costa Rica	OM669903	–
Physisporinus vinctus	JV0610_A31B-1	Mexico	JQ409460	–
Physisporinus vinctus	JV0610_A31B-2	Mexico	JQ409461	–
Picipes ailaoshanensis	Cui 12585	China	KX900068	KX900183
*Picipes ailaoshanensis**	Cui 12578	China	KX900067	KX900182
Picipes americanus	JV 0809-104	USA	KC572003	KC572042
*Picipes americanus**	JV 0509-149	USA	KC572002	KC572041
*Picipes annularius**	Cui 10123	China	KX900060	KX900176
Picipes atratus	Dai 13375	China	KX900042	KX900158
*Picipes atratus**	Cui 11289	China	KX900043	KX900159
Picipes auriculatus	Yuan 4221	China	KX900064	KX900180
*Picipes auriculatus**	Cui 13616	China	KX900063	KX900179
Picipes badius	Cui 10853	China	KU189780	KU189811
Picipes badius	Cui 11136	China	KU189781	KU189812
Picipes baishanzuensis	Cui 11395	China	KU189763	KU189794
*Picipes baishanzuensis**	Dai 13418	China	KU189762	KU189793

Species	Specimen No.	Country	ITS	LSU
Picipes brevistipitatus	Cui 11345	China	KX900074	KX900188
*Picipes brevistipitatus**	Cui 13652	China	KX900075	KX900189
Picipes cf. dictyopus	Cui 11109	China	KX900025	KX900145
Picipes cf. dictyopus	Cui 11092	China	KX900026	KX900146
Picipes conifericola	Cui 9950	China	KU189783	KU189814
*Picipes conifericola**	Dai 11114	China	JX473244	KC572061
Picipes dictyopus	TENN 59385	Belize	AF516561	AJ487945
Picipes fraxinicola	Dai 2494	China	KC572023	KC572062
Picipes fraxinicola	Wei 6025	China	KC572024	KC572063
Picipes melanopus	H 6003449	Finland	JQ964422	KC572064
Picipes melanopus	MJ 372-93	Czech	KC572026	KC572065
*Picipes nigromarginatus**	Cui 8113	China	KX900062	KX900178
Picipes pumilus	Cui 5464	China	KX851628	KX851682
Picipes pumilus	Dai 6705	China	KX851630	KX851684
Picipes rhizophilus	Dai 11599	China	KC572028	KC572067
Picipes rhizophilus	Dai 16082	China	KX851634	KX851687
Picipes subdictyopus	Cui 11220	China	KX900057	KX900173
Picipes subdictyopus	Cui 12539	China	KX900058	KX900174
Picipes submelanopus	Dai 13294	China	KU189770	KU189801
Picipes submelanopus	Dai 13296	China	KU189771	KU189802
Picipes subtropicus	Li 1928	China	KU189758	KU189790
*Picipes subtropicus**	Cui 2662	China	KU189759	KU189791
Picipes subtubaeformis	Cui 10793	China	KU189753	KU189785
*Picipes subtubaeformis**	Dai 11870	China	KU189752	KU189784
Picipes taibaiensis	Dai 5741	China	JX489169	KC572071
*Picipes taibaiensis**	Dai 5746	China	KX196783	KX196784
Picipes tibeticus	Cui 12225	China	KU189756	KU189788
*Picipes tibeticus**	Cui 12215	China	KU189755	KU189787
Picipes tubaeformis	Niemela 6855	Finland	KC572036	KC572073
Picipes tubaeformis	JV 0309-1	USA	KC572034	KC572072
Picipes ulleungus	Cui 12410	China	KX900022	KX900142
Picipes virgatus	CulTENN11219	Argentina	AF516581	AJ488122
Picipes virgatus	CulTENN11406	Argentina	AF516582	AJ488122
*Picipes wuyishanensis**	Dai 7409	China	KX900061	KX900177
Podofomes mollis	RLG6304sp	USA	JN165002	JN164791
Podofomes stereoides	Holonen	Finland	KC415179	KC415196
*Polyporus auratus**	Dai 13665	China	KX900056	KX900172
Polyporus austrosinensis	Cui 11140	China	KX900046	KX900162
*Polyporus austrosinensis**	Cui 11126	China	KX900045	KX900161
Polyporus cuticulatus	Cui 8637	China	KX851614	KX851668
Polyporus cuticulatus	Dai 13141	China	KX851613	KX851667
Polyporus guianensis	TENN 58404	Venezuela	AF516566	AJ487948
Polyporus guianensis	TENN 59093	Argentina	AF516564	AJ487947
*Polyporus hapalopus**	Yuan 5809	China	KC297219	KC297220

Species	Specimen No.	Country	ITS	LSU
Polyporus hemicapnodes	Cui 11259	China	KX851625	KX851679
Polyporus hemicapnodes	Dai 13403	China	KX851627	KX851681
Polyporus lamelliporus	Dai 12327	China	KX851622	KX851676
*Polyporus lamelliporus**	Dai 15106	China	KX851623	KX851677
Polyporus leprieurii	TENN 58579	Costa Rica	AF516567	AJ487949
*Polyporus mangshanensis**	Dai 15151	China	KX851796	KX851797
Polyporus minutissimus	**Wu 970**	**China**	**PP035829**	**PP035826**
Polyporus minutissimus*	**Wu 971**	**China**	**PP035830**	**PP035827**
Polyporus parvovarius	Yuan 6639	China	KX900049	KX900165
Polyporus parvovarius	Dai 13948	China	KX900050	KX900166
Polyporus radicatus	DAOM198916	Canada	AF516584	AJ487955
Polyporus radicatus	TENN 58831	USA	AF516585	AJ487956
Polyporus sp.*1*	Cui 11071	China	KX851642	KX851695
Polyporus sp.*1*	Cui 11045	China	KX851643	KX851696
Polyporus sp.*2*	Dai 13585A	China	KX900055	KX900171
Polyporus squamosus	Cui 10394	China	KX851635	KX851688
Polyporus squamosus	Cui 10595	China	KU189778	KU189809
Polyporus subvarius	WD2368	Japan	AB587643	AB587638
*Polyporus subvarius**	Yu 2	China	AB587632	AB587621
Polyporus tuberaster	Dai 11271	China	KU189769	KU189800
Polyporus tuberaster	Dai 12462	China	KU507580	KU507582
Polyporus umbellatus	Pen 13513	China	KU189772	KU189803
Polyporus varius	Cui 12249	China	KU507581	KU507583
Polyporus varius	Dai 13874	China	KU189777	KU189808
Pseudofavolus cucullatus	Dai 13584A	China	KX900071	KX900185
Pseudofavolus cucullatus	WD2157	Japan	AB587637	AB368114
Trametes conchifer	FP106793sp	USA	JN164924	JN164797
Trametes elegans	FP105679sp	USA	JN164944	JN164799
Trametes polyzona	Cui 11040	China	KR605824	KR605767

Notes: New sequences are in bold; "–" represents missing data; * represents type specimens

For BI, the best-fit partitioning scheme and substitution model were determined by using ModelFinder (Kalyaanamoorthy et al. 2017) via the "greedy" algorithm, branch lengths estimated as "linked" and AICc. The BI was conducted with MrBayes 3.2.6 in two independent runs, each of which had four chains for 20 million generations and started from random trees (Ronquist et al. 2012). Trees were sampled every 1,000 generations. The first 25% of the sampled trees were discarded as burn-in and the remaining ones were used to reconstruct a majority rule consensus and calculate Bayesian Posterior Probabilities (BPP) of the clades.

Phylogenetic trees were visualized using FigTree version 1.4.4 (Rambaut 2018). Branches that received bootstrap support (BS) for ML and BPPs greater than or equal to 75% (ML) and 0.95 (BPP) were considered significantly supported, respectively.

Results

Phylogenetic analyses

In the phylogenetic analysis of *Cerrena* (Fig. 1), the combined ITS1-5.8S-ITS2-nLSU dataset included sequences from 50 fungal collections representing 11 taxa, and one sample of *Irpex latemarginatus* was used as an outgroup. ModelFinder proposed models were HKY+F+G4 for ITS1, GTR+F+I+G4 for 5.8s, HKY+F+G4 for ITS2 and GTR+F+I for nLSU, for Bayesian analysis. The BI analysis resulted in an average standard deviation of split frequencies = 0.008865. As both ML and BI trees resulted in similar topologies, only the topology of the ML analysis is presented together with the statistical values of the ML (≥75%) and BPP (≥0.90) algorithms (Fig. 1). The phylogeny inferred from ITS1-5.8S-ITS2-nLSU sequences (Fig. 1) showed that our three newly sequenced samples together with three samples defined as *Cerrena* sp. 1 by Miettinen et al. (2023) formed an independent lineage with strong support (97/0.98, Fig. 1). The lineage is defined as the new species *Cerrena caulinicystidiata*.

In the phylogenetic analysis of *Polyporus* (Fig. 2), the combined ITS1-5.8S-ITS2-nLSU dataset included sequences from 113 fungal collections representing 71 species, and three samples of *Trametes* were used as outgroups. ModelFinder suggested models were GTR+F+I+G4 for ITS1, K2P+I+G4 for 5.8s, K2P+I+G4 for ITS2 and GTR+F+I+G4 for nLSU, for Bayesian analysis. The BI analysis resulted in an average standard deviation of split frequencies = 0.009675. The ML and BI trees were similar in topology, and only the topology of the ML analysis is presented along with the statistical values of the ML (≥75%) and BPP (≥0.90) algorithms (Fig. 2). The phylogeny inferred from the combined ITS1-5.8S-ITS2-nLSU sequences (Fig. 2) revealed that a new lineage with high support (100/1.00,) nests in the squamosus clade in Polyporus, namely *Polyporus minutissimus*. The new species is closely related to *P. hemicapnodes* Berk. & Broome and *P. parvovarius* H. Lee, N.K. Kim & Y.W. Lim.

Taxonomy

***Cerrena caulinicystidiata* T. Cao, F. Wu & H.S. Yuan, sp. nov.**
MycoBank No: 853719
Figs 3, 4

Holotype. China • Zhejiang Province, Hangzhou, Xiaoshan District, Yangjingwu Forest Park; 30°4'1"N, 120°19'35"E; 134 m a.s.l.; 27 Mar. 2023; on fallen angiosperm branch; F. Wu leg., Wu 661 (BJFC040654).

Etymology. *Caulinicystidiata* (Lat.): Refers to the cystidia with a tapering base.

Description. *Basidiocarps*. Annual, resupinate, sometimes effused-reflexed, continuous, easily separable, without special odor or taste when fresh, corky when dry, up to 10 cm long, 3 cm wide and 0.5 mm thick. Pore surface greyish orange to brownish orange; pores irregular, 3–8 per mm, partly split up to 2 mm long; dissepiments thin. Sterile margin finely fimbriated. Subiculum very thin, yellowish white, ca. 0.5 mm thick, a very thin brownish red crust present in the bottom next to wood. Tubes concolorous with pore surface, corky, 0.5–1 mm long.

***Hyphal structure*.** Hyphal system trimitic, generative hyphae with clamp connections; skeletal and binding hyphae CB+, IKI–; tissues unchanged in KOH.

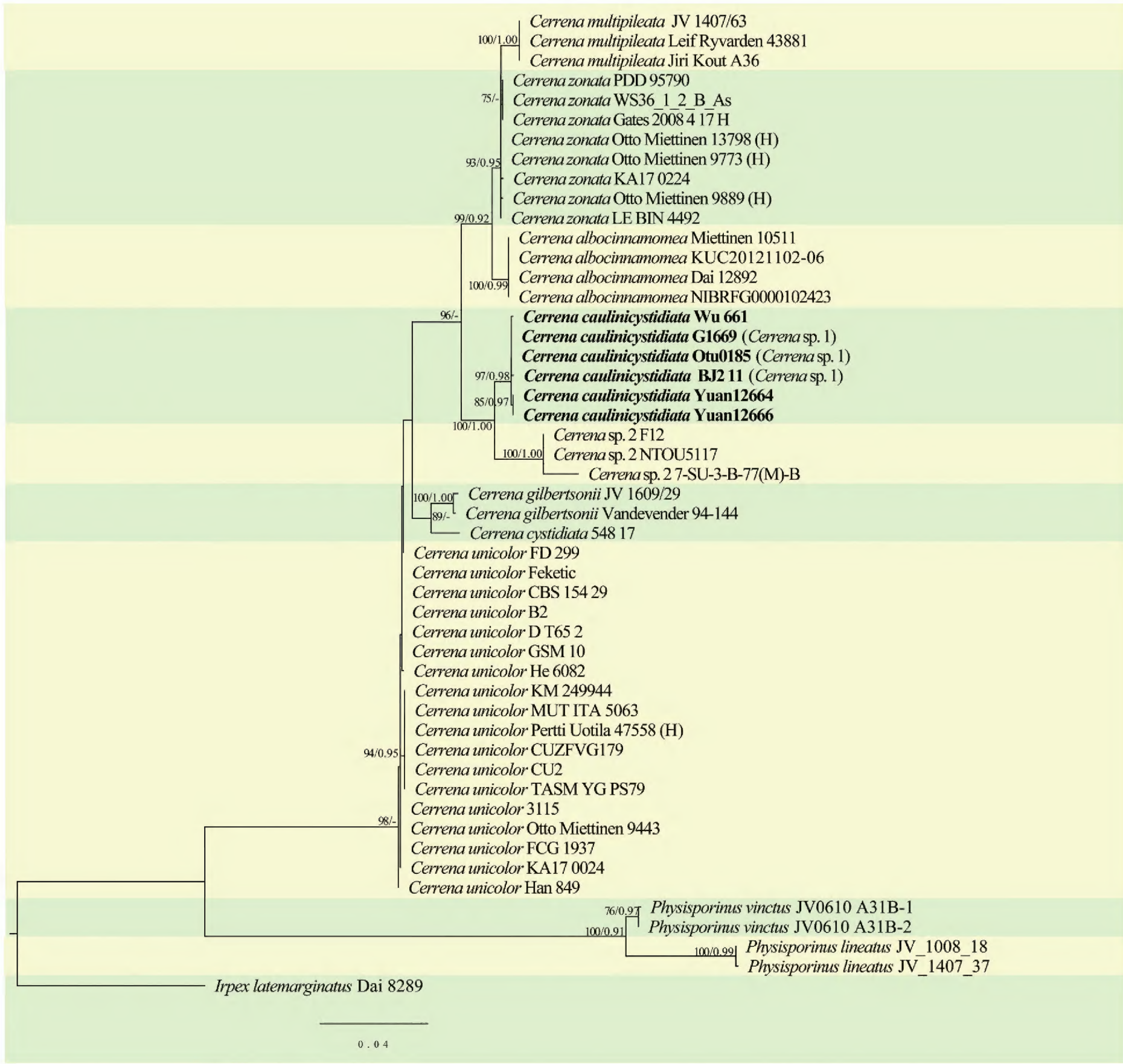
Cerrena multipileata JV 1407/63
100/1.00 Cerrena multipileata Leif Ryvarden 43881
Cerrena multipileata Jiri Kout A36
Cerrena zonata PDD 95790
75/- Cerrena zonata WS36_1_2_B_As
Cerrena zonata Gates 2008 4 17 H
Cerrena zonata Otto Miettinen 13798 (H)
93/0.95 Cerrena zonata Otto Miettinen 9773 (H)
Cerrena zonata KA17 0224
Cerrena zonata Otto Miettinen 9889 (H)
99/0.92 Cerrena zonata LE BIN 4492
Cerrena albocinnamomea Miettinen 10511
Cerrena albocinnamomea KUC20121102-06
100/0.99 Cerrena albocinnamomea Dai 12892
Cerrena albocinnamomea NIBRFG0000102423
96/- Cerrena caulinicystidiata Wu 661
Cerrena caulinicystidiata G1669 (Cerrena sp. 1)
Cerrena caulinicystidiata Otu0185 (Cerrena sp. 1)
97/0.98 Cerrena caulinicystidiata BJ2 11 (Cerrena sp. 1)
85/0.97 Cerrena caulinicystidiata Yuan12664
Cerrena caulinicystidiata Yuan12666
100/1.00 Cerrena sp. 2 F12
100/1.00 Cerrena sp. 2 NTOU5117
Cerrena sp. 2 7-SU-3-B-77(M)-B
100/1.00 Cerrena gilbertsonii JV 1609/29
89/- Cerrena gilbertsonii Vandevender 94-144
Cerrena cystidiata 548 17
Cerrena unicolor FD 299
Cerrena unicolor Feketic
Cerrena unicolor CBS 154 29
Cerrena unicolor B2
Cerrena unicolor D T65 2
Cerrena unicolor GSM 10
Cerrena unicolor He 6082
Cerrena unicolor KM 249944
Cerrena unicolor MUT ITA 5063
Cerrena unicolor Pertti Uotila 47558 (H)
94/0.95 Cerrena unicolor CUZFVG179
Cerrena unicolor CU2
Cerrena unicolor TASM YG PS79
Cerrena unicolor 3115
98/- Cerrena unicolor Otto Miettinen 9443
Cerrena unicolor FCG 1937
Cerrena unicolor KA17 0024
Cerrena unicolor Han 849
76/0.97 Physisporinus vinctus JV0610 A31B-1
100/0.91 Physisporinus vinctus JV0610 A31B-2
100/0.99 Physisporinus lineatus JV_1008_18
Physisporinus lineatus JV_1407_37
Irpex latemarginatus Dai 8289
0.04

Figure 1. Maximum Likelihood (ML) phylogenetic tree illustrating the phylogeny of *Cerrena* and related genera in five families based on the combined ITS1-5.8S-ITS2-nLSU dataset. Branches are labeled with maximum likelihood bootstrap values (ML) higher than 75% and Bayesian posterior probabilities above 0.90. The new species is given in bold.

***Subiculum*.** Generative hyphae thin- to slightly thick-walled, hyaline, clamped, frequently branched, 2–5 µm in diam; skeletal hyphae dominant, thick-walled to subsolid, unbranched, interwoven, 2.5–6 µm in diam; binding hyphae hyaline, thick-walled to subsolid, tortuose, moderately branched, 1.5–2.5 µm diam. The thin crust made up of subsolid, brownish and strongly agglutinated hyphae.

***Tubes*.** Generative hyphae infrequent, hyaline, thin- to slightly thick-walled, clamped, rarely branched, 2–3 µm diam; skeletal hyphae dominant, hyaline, thick-walled to subsolid, rarely branched, sometimes with septate, interwoven, 2–4 µm in diam; binding hyphae rare. Cystidia clavate to pyriform to ventricose, mostly thin-walled, occasionally thick-walled, smooth, 13–20 × 6–12 µm; encrusted cystidia numerous, clavate, originated from and tightly embedded in trama, 10–25 × 7–15 µm (with encrustation). Basidia short clavate, with four sterigmata and a basal clamp, 8–11 × 4–5 µm, basidioles in shape similar to basidia, but slightly smaller.

Figure 2. Maximum Likelihood (ML) phylogenetic tree illustrating the phylogeny of *Polyporus* and related genera based on the combined ITS1-5.8S-ITS2-nLSU dataset. Branches are labeled with maximum likelihood bootstrap values (ML) higher than 75% and Bayesian posterior probabilities (BPPs) more than 0.90. The new species is given in bold.

Figure 3. Basidiocarps of *Cerrena caulinicystidiata* (Holotype, Wu 661).

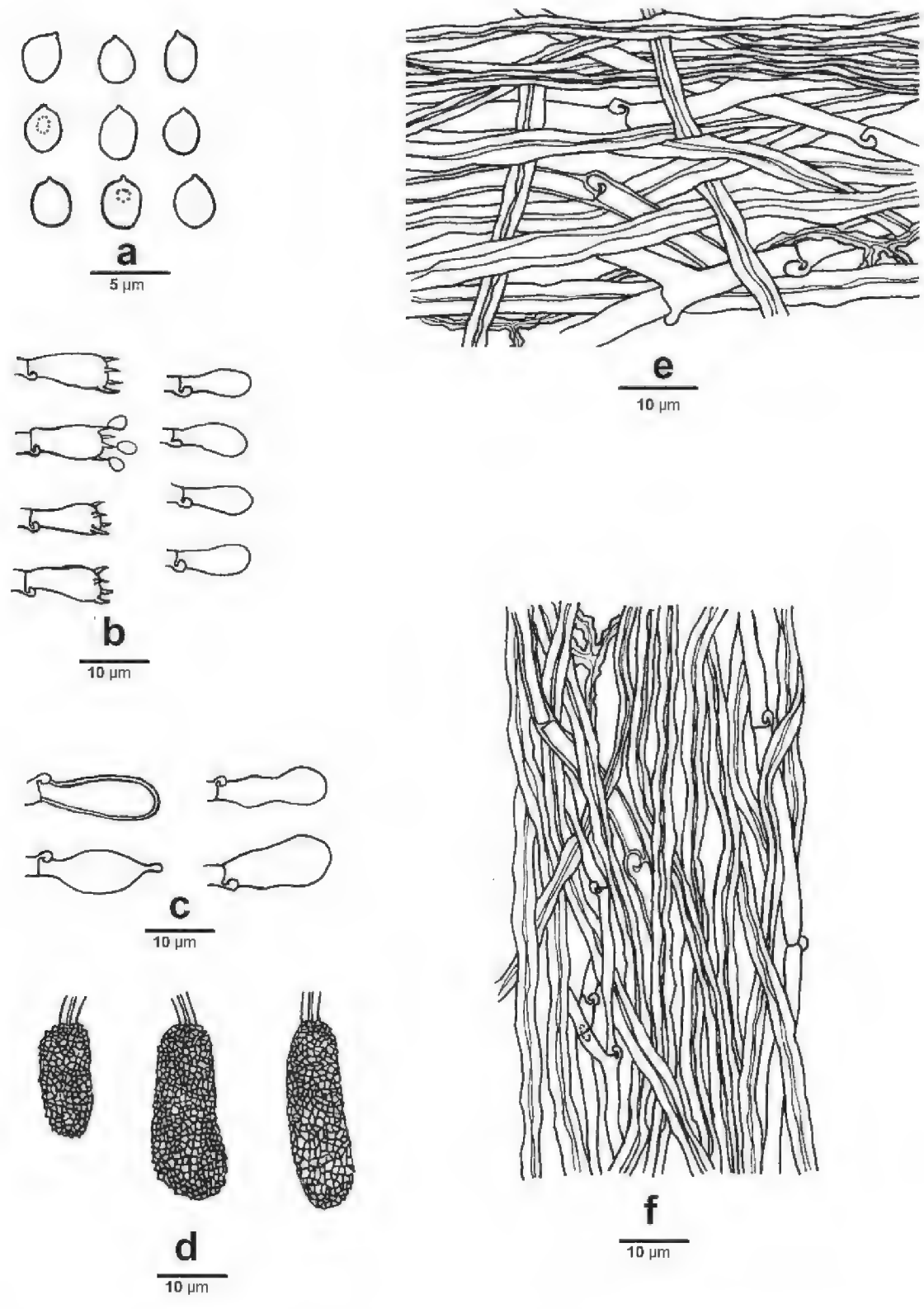

Figure 4. Microscopic structures of *Cerrena caulinicystidiata* (Wu 661) **a** basidiospores **b** basidia and basidioles **c** cystidia **d** encrusted cystidia **e** hyphae from subiculum **f** hyphae from trama.

***Basidiospores*.** Basidiospores broadly-ellipsoid to ovoid, hyaline, thin-walled, smooth, CB−, IKI−, (3−)3.2–4.5(−4.8) × (2.5−)2.8–3.5(−3.9) µm, L = 3.94 µm, W = 2.84 µm, Q = 1.38–1.44 (n = 60/2).

Additional specimens examined (paratypes). VIETNAM • Lam dong Province (Lat.), Lac Duong District, Bidoup Nui Ba National Park; 12°11'8"N, 108°40'41"E; 1495 m a.s.l.; 15 Oct. 2017; on fallen angiosperm branch; H.S. Yuan leg., Yuan 12666 (IFP 019379), Yuan 12664 (IFP 019378).

***Polyporus minutissimus* Q.Y. Zhang, Z.W. Zheng & F. Wu, sp. nov.**

MycoBank No: 853720

Figs 5, 6

Holotype. CHINA • Zhejiang Province, Hangzhou, Yuhang District, Luniao Town; 30°25'50"N, 119°42'38"E; 158.47 m a.s.l.; 9 Jun. 2023; on ground of Bamboo forest; F. Wu leg., Wu 971 (BJFC040963, holotype).

Etymology. *Minutissimus* (Lat.): Referring to the species having tiny basidiocarps.

Description. *Basidiocarps*. Annual, centrally stipitate, solitary, fleshy to soft leathery when fresh, becoming fragile when dry. Pilei flat with a depressed center or infundibuliform, up to 1.5 cm in diam and 0.5–1 mm thick. Pileal surface cream to buff yellow when dry, glabrous, occasionally zonate and with radially aligned stripes; margin sharp, incurved upon drying. Pore surface cream when dry; pores angular, 6–9 per mm; dissepiments thin, entire. Context buff cream to pale neutral when dry, fragile upon drying, up to 0.5 mm thick. Tubes white to cream when dry, decurrent, up to 0.5 mm thick. Stipe dark violet, glabrous, 0.3–0.5 cm long and 1–2 mm in diam.

***Hyphal structure*.** Hyphal system dimitic; generative hyphae bearing clamp connections, thin-walled, hyaline; skeleton-binding hyphae thick-walled with a wide lumen, with arboriform branches, IKI−, CB+; tissue unchanged in KOH.

***Context*.** Generative hyphae frequent, colorless, thin-walled, 2.5–4 µm in diam; skeleto-binding hyphae dominant, colorless, thick-walled with a wide lumen, moderately branched, strongly interwoven, 2–4.5 µm diam.

Figure 5. Basidiocarps of *Polyporus minutissimus* (Holotype, Wu 971).

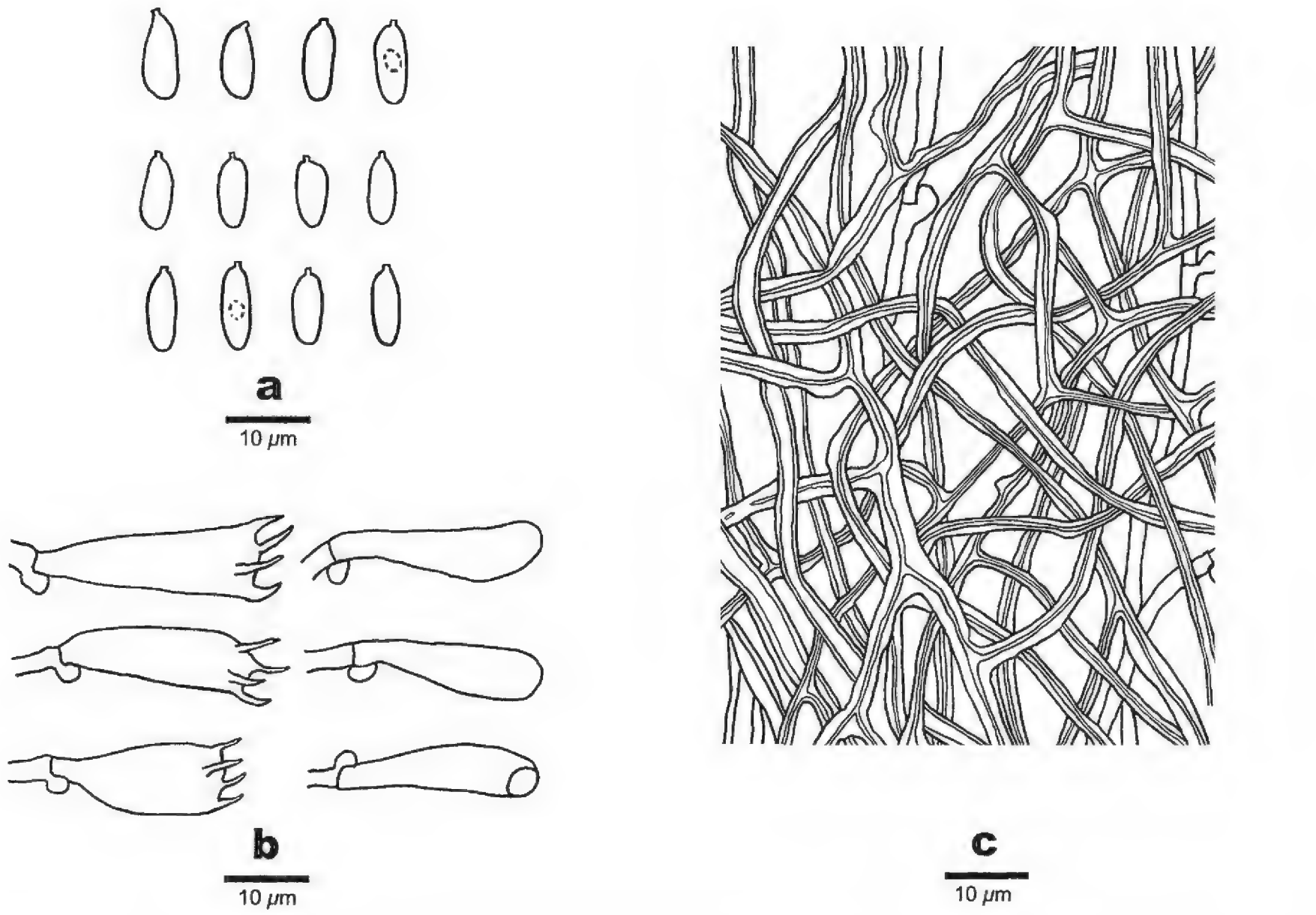

Figure 6. Microscopic structures of *Polyporus minutissimus* (Wu970/Wu 971) **a** basidiospores **b** basidia and basidioles **c** hyphae from trama.

***Tubes*.** Generative hyphae frequent, colorless, thin-walled, 2–3 μm in diam; skeleto-binding hyphae dominant, colorless, thick-walled with a wide lumen, moderately branched, interwoven, 1–3 μm in diam. Cystidia and cystidioles absent. Basidia clavate, with four sterigmata and a basal clamp connection, 22–28 × 7–9 μm; basidioles in shape similar to basidia, but slightly smaller.

***Stipe*.** Generative hyphae frequent, colorless, thin-walled, rarely branched, 3–4 μm in diam; skeleto-binding hyphae dominant, colorless, thick-walled with a wide lumen, moderately branched, interwoven, 1.5–4 μm in diam.

***Basidiospores*.** Basidiospores cylindrical to oblong, colorless, thin-walled, smooth, IKI–, CB–, 5–9.2(–10) × (2–)2.2–4(–4.2) μm, L = 7.30 μm, W = 3.23 μm, Q = 2.25–2.27 (n = 60/2).

Additional specimen examined (paratype). China • Zhejiang Province, Hangzhou, Yuhang District, Luniao Town; 30°25'50"N, 119°42'38"E; 155.11 m a.s.l; on ground of bamboo forest, 9 Jun. 2023; F. Wu leg., Wu 970 (BJFC040962).

Discussion

In this study, two new species of the Polyporales - *Cerrena caulinicystidiata* and *Polyporus minutissimus* - are proposed based on morphological and phylogenetic evidence. Our three newly sequenced *Cerrena* samples together with three samples which were defined as *Cerrena* sp. 1 by Miettinen et al. (2023) formed an independent well-supported lineage in our phylogeny (Fig. 1). The lineage is proposed as the new species *C. caulinicystidiata*. Another lineage which is defined as *Cerrena* sp. 2 in our phylogeny is closely related to

C. caulinicystidiata, but we didn't collect one specimen within the lineage, so the lineage is considered to be *Cerrena* sp.

Cerrena caulinicystidiata is characterized by its resupinate, sometimes effused-reflexed basidiocarps, greyish orange to brownish orange pore surface, 3–8 per mm pores, and subglobose basidiospores, 3.2–4.5 × 2.8–3.5 µm in size. *C. albocinnamomea* (Y.C. Dai & Niemelä) H.S. Yuan originally described from Northeast China resembles *C. caulinicystidiata* by sharing resupinate and easily separable basidiocarps. However, *C. albocinnamomea* differs from *C. caulinicystidiata* by its clavate to pyriform cystidia, slightly smaller ellipsoid basidiospores (2.8–3.5 × 2–3 µm vs. 3.2–4.5 × 2.8–3.5 µm), and a dimitic hyphal system (Yuan 2014).

In addition, *Rigidoporus vinctus* (Berk.) Ryvarden [≡ *Physisporinus vinctus* (Berk.) Murrill, Wu et al. 2017] resembles *C. caulinicystidiata* by having resupinate basidiocarps, ochraceous pore surface, ventricose cystidia with a subcylindric appendage, encrusted cystidia, and subglobose basidiospores, but it can be distinguished from the latter species by its smaller pores (6–12 per mm vs. 3–8 per mm) and generative hyphae with simple septa (Ryvarden and Johansen 1980).

The genus *Cerrena* is widely distributed and has diverse morphological characteristics. Currently, there are 13 records according to Index Fungorum (http://www.indexfungorum.org). However, *C.* '*gilbertsonii*' Ryvarden cannot be distinguished from *C. cystidiata* Rajchenb. & De Meijer by morphological characteristics, and *C.* '*multipileata*' (C.L. Leite & J.E. Wright) Miettinen cannot be distinguished from *C. zonata* (Berk.) H.S. Yuan (Miettinen et al. 2023). *Cerrena aurantiopora* J.S. Lee & Y.W. Lim is a synonym of *C. albocinnamomea* (Lee and Lim 2010; Miettinen et al. 2023). Therefore, we provide a Key to 11 undisputed *Cerrena* species including the new species.

Key to species of the *Cerrena*

1	Paleotropical or temperate-boreal species	**2**
–	Neotropical (South American) species	**9**
2	Basidiocarp poroid, occasionally lacerate	**3**
–	Basidiocarp irpicoid	**8**
3	Pore surface umbrinous to bay or blackish	***C. subglabrescens***
–	Pore surface white, light orange to brown	**4**
4	Pores umber, round, 1–2 per mm	***C. drummondii***
–	Pores round to angular, > 3 per mm	**5**
5	Basidiospores narrowly ellipsoid, 7.5–10 × 2.5–3.5 µm	***C. caperata***
–	Basidiospores ellipsoid to broadly-ellipsoid, < 6 µm in length	**6**
6	Pores angular, dissepiments even or lacerate	***C. albocinnamomea***
–	Pores rounded to irregular	**7**
7	Basidiocarps coriaceous, imbricate	***C. fulvocinerea***
–	Basidiocarps resupinate, sometimes effused-reflexed	***C. caulinicystidiata***
8	Pore surface white to cream	***C. unicolor***
–	Pore surface first white to pale ochraceous	***C. zonata***
9	Cystidia present	***C. cystidiata***
–	Cystidia absent	**10**
10	Pore surface pale cinnamon to brown	***C. sclerodepsis***
–	Pore surface dark brown to almost black	***C. hydnoides***

In the phylogenetic analysis of *Polyporus*, *P. minutissimus* was assigned to the squamosus clade with strong support (100/1.00, Fig. 2). The squamosus clade has always been supported by phylogenetic analysis based on the ITS+nLSU or eight-gene datasets, but the species within this clade cannot be combined into a monophyletic genus because they manifest greatly diverse morphology (Ji et al. 2022). Phylogenetically, *P. minutissimus* is closely related to *P. hemicapnodes* and *P. parvovarius* (Fig. 2). *P. hemicapnodes* was described from Dolosbagey (Sri Lanka). For some time, it was treated as a synonymy of *P. leprieurii* (Núñez and Ryvarden 1995), which differs from *P. minutissimus* by its larger basidiocarps (up to 10 cm vs. up to 1.5 cm), cream to tan pore surface, and longer stipe (up to 5 cm vs. up to 0.5 cm, Berkeley and Broome 1873). *Polyporus parvovarius* has microscopic features similar to *P. minutissimus*. However, *P. parvovarius* differs by its smaller basidiocarps (up to 0.35 cm vs. up to 1.5 cm) and light buff to brown pileal surface (Tibpromma et al. 2017).

Macro-morphologically, *Polyporus minutissimus* has a depressed center or infundibuliform basidiocarps and black stipe, cream to buff yellow pileal surface, and 6–9 per mm pores. Microscopically, it has a dimitic hyphal system, strongly branched skeleton-binding hyphae in both trama and context, and cylindrical basidiospores. Morphologically, *P. lamelliporus* B.K. Cui, Xing Ji & J.L. Zhou is similar to *P. minutissimus* by sharing depressed center or infundibuliform basidiocarps, cream to buff yellow pileal surface, and similar-sized basidiospores, but the former differs through its larger basidiocarps (up to 5.2 cm vs. up to 1.5 cm), longer stipe (1–3.5 cm vs. 0.3–0.5 cm), and larger pores (0.5–1 per mm vs. 6–9 per mm, Ji et al. 2022). In addition, *Picipes baishanzuensis* J.L. Zhou & B.K. Cui, which is similar to *P. minutissimus* and shares infundibuliform basidiocarps and a black stipe, has also been reported from Baishanzu nature reserve, which is the type producing area of our new species. However, *P. baishanzuensis* differs from *P. minutissimus* by its larger basidiocarps (up to 5.5 cm vs. up to 1.5 cm) and smaller basidiospores (6.6–7.9 × 2.5–3.1 µm vs. 5–9.2 × 2.2–4 µm; Zhou et al. 2016).

Polyporus is a very complicated genus with more than 3000 records according to the Index Fungorum. However, studies on *Polyporus* species in China are gradually being carried out, with some Chinese species having been described in Cui et al. (2019) and Ji et al. (2022). Therefore, we provide a Key to Chinese *Polyporus* species including the new species.

Key to species of *Polyporus* in China

1 Stipe absent ..... ***P. megasporoporus***
– Stipe present **2**
2 Stipe bearing black cuticle **3**
– Stipe white to ochraceous **7**
3 Pileal surface covered with dark-brown to reddish-brown squamules ..... ***P. squamosus***
– Pileal surface glabrous **4**
4 Pores more than 5 per mm **5**
– Pores less than 5 per mm **6**
5 Pileal surface concentrically zonate; basidiospores 5.4–7.6 × 2.9–3.8 µm ..... ***P. hemicapnodes***
– Pileal surface azonate; basidiospores 7.5–9 × 2.5–3.3 µm ..... ***P. varius***

6	Pores 3–5 per mm	***P. mangshanensis***
–	Pores 1–2 per mm	***P. subvarius***
7	Stipes numerous and branched	***P. umbellatus***
–	Stipes usually single and not branched	**8**
8	Basidiospores < 8 µm in length	**9**
–	Basidiospores > 8 µm in length	**11**
9	Basidiocarps imbricate	***P. hapalopus***
–	Basidiocarps solitary	**10**
10	Pores angular, 2–3 per mm	***P. brumalis***
–	Pores round, 4–5 per mm	***P. ciliatus***
11	Pileal surface with radial stripes	**12**
–	Pileal surface without radial stripes	**13**
12	Pores 2–5 per mm	***P. cuticulatus***
–	Pores 6–9 per mm	***P. minutissimus***
13	Basidiospores usually < 10 µm in length	**14**
–	Basidiospores usually > 10 µm in length	***P. tuberaster***
14	Cystidioles absent	**15**
–	Cystidioles infrequent	***P. austrosinensis***
15	Basidia < 27 µm in length	**16**
–	Basidia > 27 µm in length	***P. lamelliporus***
16	Basidiospores smaller, 6–8.3 × 2.2–3 µm	***P. arcularius***
–	Basidiospores larger, 7.7–10 × 3–3.9 µm	***P. auratus***

Polyporales is a large group of Basidiomycota with diverse morphology and phylogeny. There have been over 577 taxonomic proposals in the Polyporales and 2,183 publications with the keyword 'Polyporales' over the past decade (Binder et al. 2013; Justo et al. 2017). However, the species in the order are still not sufficiently investigated in Asia, especially in the subtropics and tropics (Li et al. 2016; Hyde 2022). New DNA sequencing techniques have revolutionized fungal taxonomy and diversity, with multi-marker datasets. In the present study, two new polypore species, *C. caulinicystidiata* and *P. minutissimus* were found in subtropical regions, which enriches our understanding of the fungal diversity of the Polyporales in Asia.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Data curation: LRZ. Investigation: HSY, QYZ, FW, ZWZ. Methodology: QYZ, ZWZ. Resources: HSY. Supervision: LRZ, FW. Validation: QYZ, ZWZ. Writing - original draft: ZWZ, QYZ. Writing - review and editing: FW.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

31102 Treebase

Authors: Zi-Wei Zheng, Qiu-Yue Zhang, Li-Rong Zhang, Hai-Sheng Yuan, Fang Wu

Data type: nxs

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Supplementary material 2

31103 Treebase

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Data type: nxs

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